



Acta Botanica  
Mexicana

# Morphology, ultrastructure, and molecular phylogeny of the marine planktonic phytoflagellate *Heterosigma akashiwo* (Raphidophyceae, Heterokontophyta) from the tropical Pacific Ocean, Mexico

## Morfología, ultraestructura y filogenia molecular del fitoflagelado planctónico marino *Heterosigma akashiwo* (Raphidophyceae, Heterokontophyta) del Océano Pacífico tropical, mexicano

David U. Hernández-Becerril<sup>1,3</sup> , Jovanny Arana-García<sup>2</sup> , Alexis Escarcega-Bata<sup>2</sup> 

### Abstract:

**Background and Aims:** Marine planktonic phytoflagellates of the class Raphidophyceae (Heterokontophyta) comprise a small group of species characterized by medium-sized, fragile, solitary and motile cells, mostly photosynthetic, important primary producers and capable of forming harmful algal blooms (HAB) and producing several substances harmful to fishes. In this work, one strain of the potentially ichthyotoxic raphidophyte *Heterosigma akashiwo* from one location of the tropical Mexican Pacific was established and studied.

**Methods:** The morphology and ultrastructure of the species were studied in detail, using Light Microscopy (LM) and Transmission Electron Microscopy (TEM). Additionally, molecular phylogenies, based on LSU rDNA sequences, were performed.

**Key results:** Our findings were consistent with previous morphological descriptions: mobile, solitary, naked and fragile cells with a high morphological variability, from ovoid or oblong to spheroidal shape, slightly flattened dorsiventrally, two unequal flagella, numerous golden-brown chloroplasts, and one centrally located nucleus. Ultrastructural details showed organelles of the cells such as nucleus, nucleolus, chloroplasts, pyrenoids, plastoglobules, mitochondria, flagella, mucocysts and vacuoles. Our findings evidenced some differences with previous studies, such as size and shape of nucleus and chloroplasts and their pyrenoids, and the detection of plastoglobules. The molecular phylogeny showed that the sequence obtained in this study coincided in the clade formed by sequences of the species from other areas in the Atlantic and Pacific Oceans from both hemispheres, with 100% support.

**Conclusions:** This is the first study of the morphology and ultrastructure of *Heterosigma akashiwo* from the tropical Mexican Pacific, showing the general and particular features as in other parts of the world, and molecular phylogeny confirmed the morphological identification. The species displays certain ecological features which can provide advantages in producing blooms. The species is widespread globally and broadly distributed in coastal areas of the Mexican Pacific.

**Key words:** Mexican Pacific, morphology, phylogeny, phytoplankton, ultrastructure.

### Resumen:

**Antecedentes y Objetivos:** Los fitoflagelados planctónicos marinos de la Clase Raphidophyceae (Heterokontophyta) comprenden un pequeño grupo de especies caracterizadas por células de tamaño mediano, frágiles, solitarias y móviles, fotosintéticas, productores primarios importantes, y capaces de formar proliferaciones algales nocivas (PAN) y producir sustancias nocivas a los peces. En este trabajo se estableció y estudió una cepa de la raphidophyte potencialmente ictiotóxica *Heterosigma akashiwo* de una localidad del Pacífico tropical mexicano.

**Métodos:** La morfología y ultraestructura de la especie se estudiaron con detalle, utilizando Microscopía de Luz (ML) y Microscopía Electrónica de Transmisión (MET). Adicionalmente, se elaboraron filogenias moleculares basadas en secuencias de LSU rADN.

**Resultados clave:** Nuestros hallazgos fueron consistentes con descripciones morfológicas previas: células solitarias, móviles, desnudas y frágiles con gran variabilidad, formas ovóides, oblongas a esferoides, ligeramente comprimidas dorsoventralmente, con dos flagelos desiguales, numerosos cloroplastos dorado-café, y un núcleo localizado en el centro. Los detalles ultraestructurales mostraron organelos de la célula como núcleo, nucléolo, cloroplastos, pirenoides, plastoglobulos, mitocondrias, flagelos, mucocistos y vacuolas. Algunas diferencias con estudios anteriores fueron evidentes, como la forma y el tamaño del núcleo, los cloroplastos y sus pirenoides, y la detección de plastoglobulos. La filogenia molecular demostró que las secuencias obtenidas en este estudio se agruparon en el clado formado por secuencias de la especie en otras áreas de los Océanos Atlántico y Pacífico de ambos hemisferios con 100% de soporte.

**Conclusiones:** Este es el primer estudio de la morfología y ultraestructura de *Heterosigma akashiwo* del Pacífico tropical mexicano, que ha mostrado sus características generales y particulares como en otras regiones del mundo, mientras que la filogenia molecular confirmó la identidad morfológica de la especie. La especie exhibe ciertas características ecológicas que provee ventajas para la formación de proliferaciones. Se distribuye ampliamente en los mares de todo el mundo, así como en áreas costeras del Pacífico mexicano.

**Palabras clave:** filogenia, fitoplancton, morfología, Pacífico mexicano, ultraestructura.

<sup>1</sup>Universidad Nacional Autónoma de México, Instituto de Ciencias del Mar y Limnología, Ciudad Universitaria, Coyoacán, 04510 Cd. Mx., Mexico.

<sup>2</sup>Universidad Autónoma Metropolitana-Iztapalapa, Departamento de Hidrobiología, Iztapalapa, 09340 Cd. Mx., Mexico.

<sup>3</sup>Author for correspondence: dhernand@cmarl.unam.mx

Received: January 19, 2026.

Reviewed: April 23, 2026.

Accepted by Marie-Stéphanie Samain: July 29, 2026.

Published Online first: August 25, 2026.

Published: Acta Botanica Mexicana 133 (2026).



This is an open access article under the Creative Commons 4.0 Attribution-NonCommercial Licence (CC BY-NC 4.0 International)

To cite as: Hernández-Becerril, D. U., J. Arana-García and A. Escarcega-Bata. 2026. Morphology, ultrastructure, and molecular phylogeny of the marine planktonic phytoflagellate *Heterosigma akashiwo* (Raphidophyceae, Heterokontophyta) from the tropical Pacific Ocean, Mexico. Acta Botanica Mexicana 133: e2560. DOI: <https://doi.org/10.21829/abm133.2026.2560>

e-ISSN: 2448-7589

## Introduction

Planktonic golden-brown phytoflagellates belonging to the Raphidophyceae (Heterokontophyta) are important primary producers, usually occurring as solitary and motile cells, with high potential to produce harmful algal blooms (HAB), especially in coastal marine waters (Throndsen, 1997; Hallegraeff and Hara, 2004; Horiguchi, 2017). The cells are medium to large sized (20-80 µm in length), monads with no rigid covering or wall, two typical heterokont (heterodynamic) flagella arising from the apex of the cells, and numerous green, yellow or golden chloroplasts (Jeffrey and Vesk, 1997; Throndsen, 1997; Hallegraeff and Hara, 2004; Horiguchi, 2017; Mehdizadeh Allaf, 2023). Mucocysts are present in the members of this group, and the photosynthetic pigments include chlorophylls *a*, *c*<sub>1</sub> + *c*<sub>2</sub>, fucoxanthin, violaxanthin and β, β-carotene (Jeffrey and Vesk, 1997). The cells are extremely fragile and are not usually recognizable in preserved phytoplankton field samples (Throndsen, 1997; Hallegraeff and Hara, 2004; Horiguchi, 2017).

This group contains a reduced number of genera (10) and species, truly photosynthetic, with representatives in marine and freshwater environments (Horiguchi, 2017). There are reports of mixotrophic behavior among raphidophytes (Jeong, 2011). Production of “reactive oxygen species” (ROS), mucus secretion, toxin production and hemolytic activity are attributed to members of these microalgae (Yang et al., 1995; Khan et al., 1997; Twiner and Trick, 2000; Marshall et al., 2005; Dorantes-Aranda et al., 2015; Horiguchi, 2017).

*Heterosigma akashiwo* (Hada) Hada ex Y.Hara & Chihara is a raphidophyte of worldwide distribution with high potential to form HAB and kill fish (Smayda, 1998; Hallegraeff and Hara, 2004; Lassus et al., 2016; Gómez et al., 2022; Anderson et al., 2021; Mehdizadeh Allaf, 2023). The species produces allelopathic substances (protein-polysaccharide complex), causing changes in species diversity (Mehdizadeh Allaf, 2023). Regarding fish kills, the toxic mechanism is not well known, but it includes both gills clogged by mucus and gill-cells destruction by hemolytic substances (Chang et al., 1990) and neurotoxin production (Khan et al., 1997). Hemolytic effects on fish gill-cells are assumed to be due to the secretion of ROS (Twiner and Trick, 2000; Lassus et al., 2016).

The genus *Heterosigma* Hada ex Y.Hara & Chihara has historically been well-known and studied, and traditionally used to encompass only one recognized species, *Heterosigma akashiwo*, although more recently another species was described, *Heterosigma minor* Tomas, Engesmo & Eikrem (Engesmo et al., 2016). The latter species was found to be morphologically smaller, rounder and with fewer mucocysts than *H. akashiwo* (Engesmo et al., 2016), and, according to its secondary structure of ITS2, *Heterosigma minor* is sister to *H. akashiwo* (Engesmo et al., 2016).

Most probably, the first report of raphidophyte microalgae in the plankton from Mexican coastal waters was made by Hernández-Becerril and Bravo-Sierra (1999), where the species *Heterosigma akashiwo* was successfully cultured, identified and described. Later, Cortés-Lara et al. (2003) detected high cell densities ( $4\text{--}6 \times 10^6$  cells l<sup>-1</sup>) of *Fibrocapsa* cf. *japonica* in one small bay in the central Mexican Pacific, whereas Bravo-Sierra (2004) included the raphidophycean algae in his approach of the potentially toxic and harmful phytoflagellates from Mexican waters. A more complete account of the group was provided by Band-Schmidt et al. (2004), where the species *Chattonella marina* (Subrahmanyam) Y.Hara & Chihara, *Fibrocapsa japonica* S.Toriumi & H.Takano and *Heterosigma akashiwo* were recognized, described and illustrated from cultures established from several spots of different locations from both the Gulf of Mexico (Atlantic Ocean) and the Mexican Pacific. Since then, numerous reports of various raphidophyte species have been made, in coastal waters of the Mexican Pacific, especially the Gulf of California (Band-Schmidt et al., 2005; Martínez-López et al., 2006; Gárate-Lizárraga et al., 2009), even recording “marine die-offs” due to blooms of *Chattonella marina* and *Chattonella marina* cf. *ovata* (Barraza-Guardado et al., 2004; Cortés-Altamirano et al., 2006). *Heterosigma akashiwo* was also included in the review of the toxic and harmful marine phytoplankton and microalgae in Mexican coasts (Hernández-Becerril et al., 2007).

More recently, studies on pigment content, toxicity and molecular phylogeny of the species *Chattonella subsalsa* B.Biecheler, *Chattonella marina* and *Fibrocapsa japonica* were made, confirming additionally the occurrence of *Chattonella subsalsa* in the Gulf of California (Band-Schmidt et al., 2012). A review of the raphidophyte



microalgae of the marine phytoplankton in Mexico was provided by Hernández-Becerril (2014), and another review of the group in the Gulf of California was made by López-Cortés et al. (2016). Finally, Meave del Castillo and Zamudio-Reséndiz (2018) cited *Chattonella antiqua* (Hada) C. Ono and *Fibrocapsa japonica* as species potentially forming HAB in Acapulco Bay, in a more tropical zone of the southern Mexican Pacific.

The detailed study of *Heterosigma akashiwo*, a prominent member of the Raphidophyceae, with a global distribution, especially its morphological variability, ultrastructural characteristics and its recognition by molecular phylogeny, had not been previously made in waters of the Mexican Pacific. Therefore, the aim of this work was to study the morphology (including the morphological variation), ultrastructure and molecular phylogeny of *Heterosigma akashiwo* based on cultures established from a location in the Acapulco Bay, a tropical area of the southern Mexican Pacific, to provide a complete characterization of the species in that area.

## Material and Methods

### Sampling and culture establishment

Surface samples were collected using a Van Dorn bottle (3l) in the 'Parque de la Reina' station, in Acapulco Bay, southern Mexican Pacific (16°50'58.8"N, 99°54'1.6"W) (Fig. 1) in November 2021. Temperature was 29 °C and salinity 35 PSU (Practical Salinity Units). One live sample was protected from light and transported in a cold container to the laboratory (Laboratorio de Diversidad y Ecología del Fitoplancton Marino, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México) for further analysis. The un-fixed sample was filtered with a 20 µm pore size mesh and then passively concentrated by gravity using a 3 µm membrane filter. The cells of interest were isolated under an inverted microscope (Invertoskop Zeiss, Jena, Germany) using sterile micropipettes and placed in a 96-well plate pre-filled with IMK culture medium (Daigo's, Wako, Tokyo, Japan). Culture conditions were temperature 24 °C, salinity 35 PSU, and a 12:12 light/darkness photoperiod.

### Microscopy

Observations of living cells of one strain (HAC6) were carried out with a bright field light microscope (Olympus BX40F-3,

Tokyo, Japan), at 40× and 100×. Cell movements, measurements and micrographs were obtained with a camera Canon G10 (Tokyo, Japan).

### Ultrastructure

TEM samples were prepared according to Papini et al. (2017) and Benico et al. (2019). Two milliliters of exponentially growing culture of strain HAC6 were pre-fixed in glutaraldehyde (2% final concentration) and post-fixed in osmium tetroxide (OsO<sub>4</sub>) (1% final concentration) for 8 h, followed by dehydration using a series of ethanol solutions (30%, 50%, 70%, 90%, 95% and 100%). They were then embedded in low-viscosity resin, sectioned, and stained with uranyl acetate and lead citrate. Observations were made using a JEOL-JEM-1200 (Tokyo, Japan) at an acceleration voltage of 100 kV.

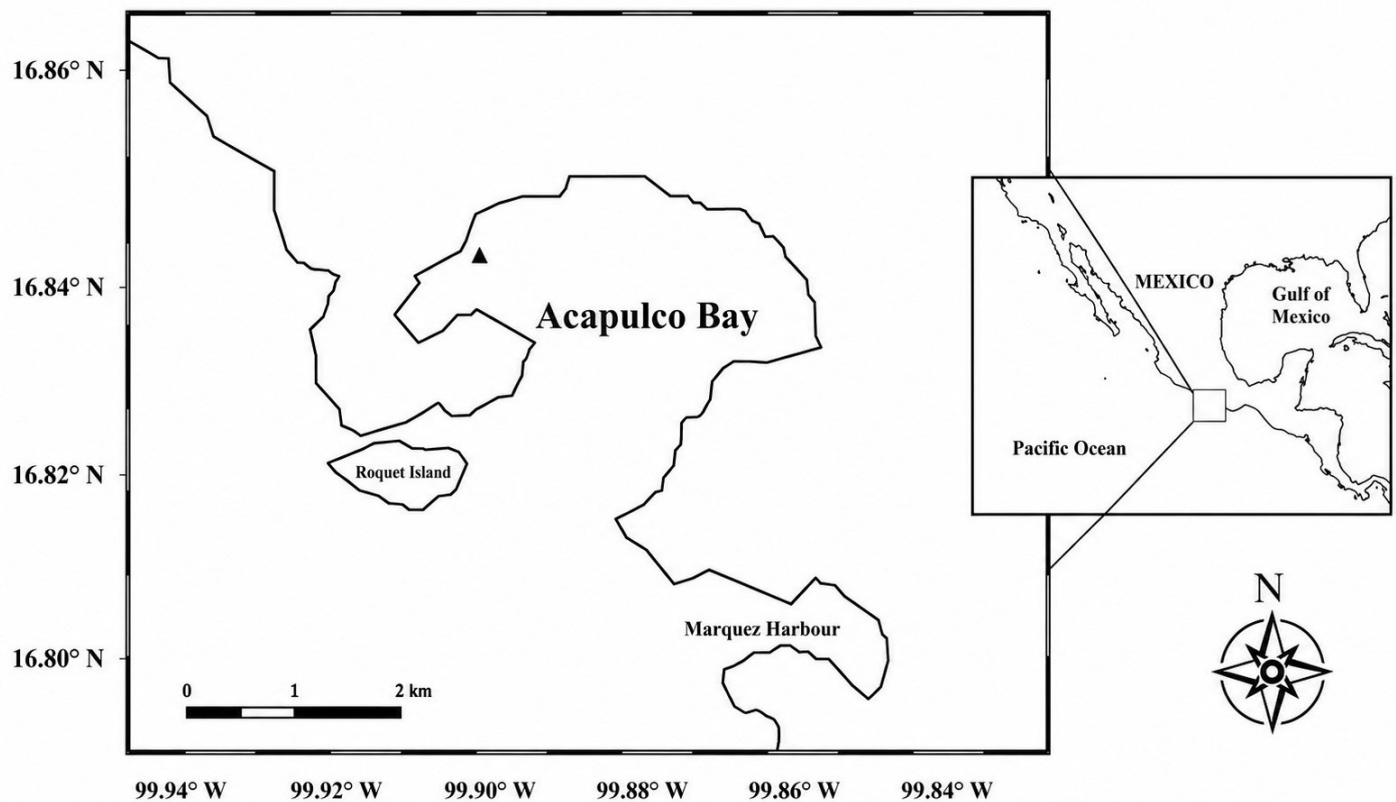
### DNA extraction, PCR and sequencing

DNA was extracted using a commercial kit (Zymo Research, USA), following the manufacturer's instructions. For PCR amplification, partial LSU rDNA sequences, the primers D1R-F (5'-ACCCGCTGAATTTAAGCATA-3') and D2C-R (5'-CCTTGGTCCGTGTTTCAAGA-3') (Scholin et al., 1994) as well as a commercial master mix (PROMEGA GoTaq<sup>®</sup> G2 Hot Start) were used. The thermocycling conditions (BIO RAD T100, California, USA) were as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min (Escarcega-Bata et al., 2021). The PCR products were confirmed via electrophoresis of 1% agarose gels. Sequencing and purification (Sequencer iSeq100 Illumina, California, USA) were performed in the genomic sequencing laboratory of the Instituto de Biología, Universidad Nacional Autónoma de México (Mexico City).

### Alignment and phylogenetic analysis

Sequences were aligned in BioEdit v. 7.0.5 (Hall, 1999) with those of a selected group of species of the order Raphidophyceae, obtained from GenBank of the National Center for Biotechnology Information. The diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin was used as the outgroup. Usually, members of Heterokonto-





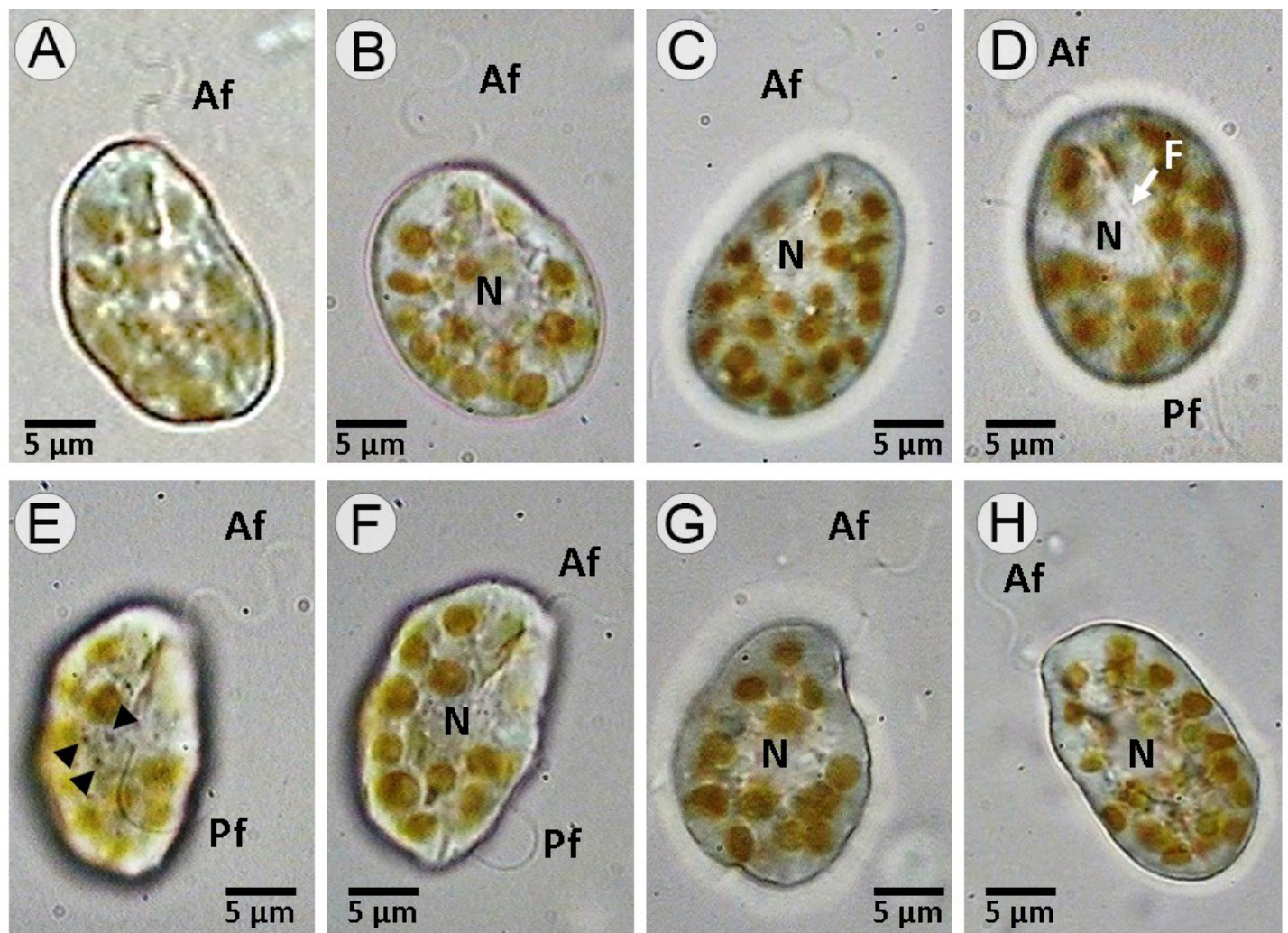
**Figure 1:** Location of the study area, Acapulco Bay, southern Mexican Pacific. Drawings by Jovanny Arana-García.

phyta (including Bacillariophyceae -diatoms-, Dictyochophyceae -silicoflagellates and others-, and other Raphidophyceae) have been used as outgroup in the molecular phylogenies of *Heterosigma akashiwo* and most raphidophytes (Engesmo et al., 2016; Flores-Leñero et al., 2022; Lum et al., 2022) (Appendix). The phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) for the 28S marker. The ML analysis was performed in RAxML (Stamatakis, 2006). Support for each branch was determined from 1000 bootstrap replicates. The BI analysis was performed in MrBayes v. 3.2.2 (Ronquist et al., 2012). The evolutionary model GTR (general time reversible) was selected based on the Maximum Likelihood ratio test, using the jModeltest software (Posada, 2008). Four Markov chain Monte Carlo chains were used, starting with a random tree and sampling every 1000 iterations for  $1 \times 10^8$  generations, discarding the first 25% of trees (chain warm-up). Pairwise distance values (p-distances), Jukes-Cantor corrected distances were calculated in MEGA v. 11 (Tamura et al., 2011).

## Results

### Description of the species

In LM we observed vegetative cells solitary and motile, naked and fragile, and quite variable in shape and size. The cells are ovoid or oblong to spheroidal, slightly flattened dorsiventrally, 18-26  $\mu\text{m}$  in length, 11-16  $\mu\text{m}$  in width ( $n=48$ ) (Fig. 2A-H). The cells have no rigid covering such as theca or wall. Usually, each cell has two unequal, heterodynamic flagella which are inserted in an antero-lateral furrow close to the apex (Fig. 2C, D). One flagellum is directed anteriorly and moves actively, whereas the other (posterior one) is rigid and has occasional and slow movement (Fig. 2D-F). The furrow extends and crosses obliquely the ventral part of cell, located 1/4-1/3 of the cell length ending about the middle of each cell (Fig. 2D). There are 14-24 discoid, brown to yellow chloroplasts per cell, located in the periphery, and a round to slightly elongate nucleus, centrally situated (Fig. 2B-H). Some scattered mucocysts were occasionally detected (Fig. 2E). No small, resting cells or cysts were found.



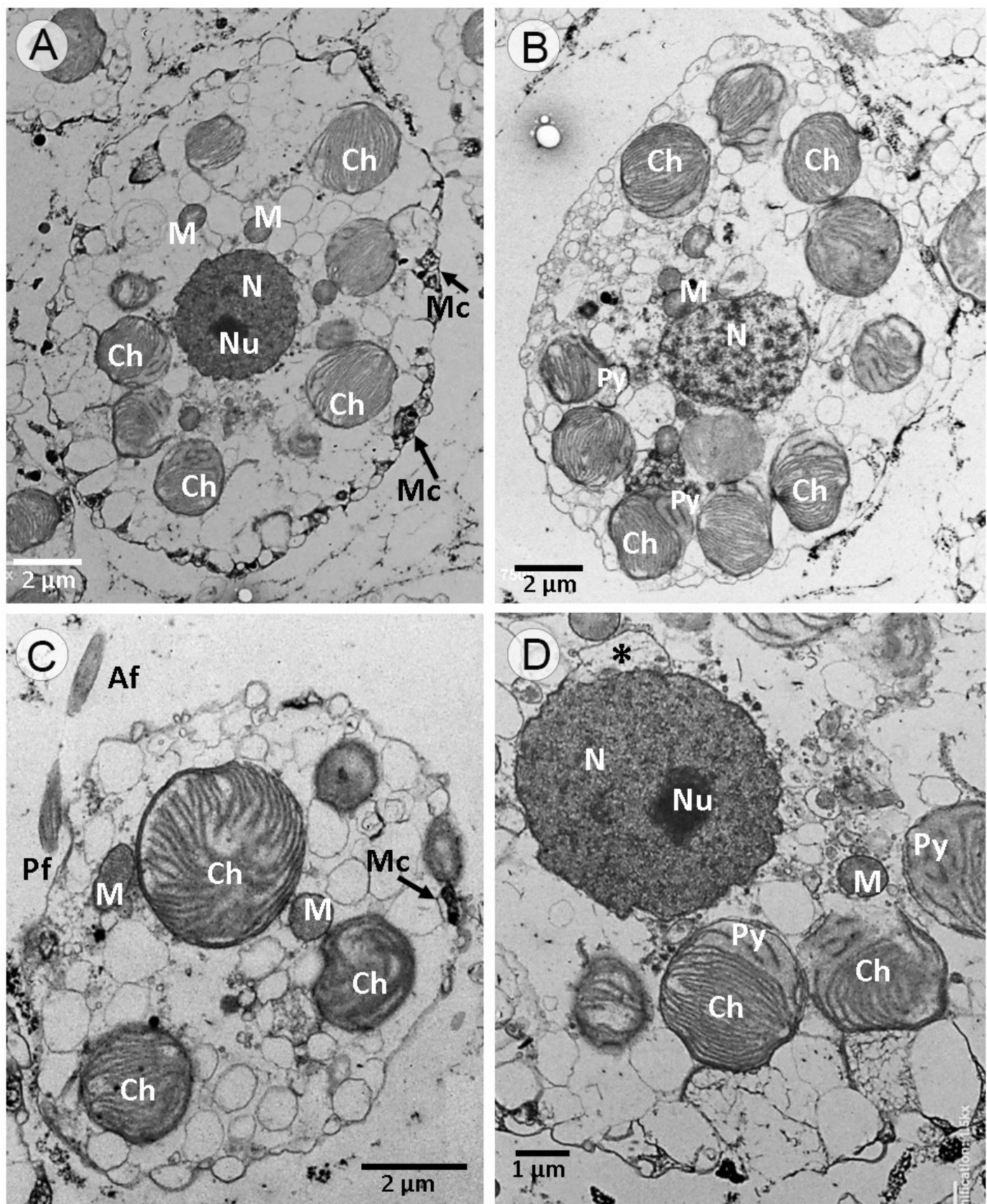
**Figure 2:** *Heterosigma akashiwo* (Hada) Hada ex Y.Hara & Chihara, morphological variability of the species in LM. A-D. several different alive specimens from culture show many round, brown to yellow chloroplasts, centrally located nucleus, anterior and posterior flagella and furrow; E, F. one specimen in two different moments, showing the two flagella and mucocysts (arrowheads); G, H. two other cells with different shapes. Af=anterior flagellum, F=furrow, N=nucleus, Pf=posterior flagellum. All micrographs by David U. Hernández-Becerril.

### Ultrastructure

Irregularly shaped cells were observed as in LM, ovoid to oblong, with no thick membrane or rigid covering, but with extrusomes (Fig. 3A-C). Numerous small to medium-sized and nearly round chloroplasts in the periphery of the cells, and relatively large and rounded to oblong nucleus, centrally located, some nuclei additionally showed a nucleolus (Fig. 3A-D). Several mitochondria were present, mainly distributed around the nucleus (Fig. 3A-D). Sometimes, dense granules at the periphery, close to the cell membrane, were found and interpreted as mucocysts (Fig. 3A, C). The two flagella (anterior and posterior, respectively) were occasionally seen in section (Fig. 3C, 4E).

The nuclei were medium to relatively large, irregularly circular, although some were oblong, situated in the center of the cell (Fig. 3A, B, D). The nucleolus was conspicuous, although it was not always observed in the nuclei, and there was only one nucleolus per nucleus (Fig. 3A, B, D); a structure interpreted as a nucleus blebbing was detected (Fig. 3D, asterisk).

The chloroplasts appeared almost circular, showing not very differentiated pyrenoids, directed to the center of the cell (Fig. 3A-D, 4A, B). These chloroplasts were surrounded by two membranes of the chloroplast envelope and two membranes of the chloroplast endoplasmic reticulum (Fig. 4A, B). The thylakoids were arranged in groups of three, and additionally very tiny spherical plastoglobules were also detected among



**Figure 3:** *Heterosigma akashiwo* (Hada) Hada ex Y.Hara & Chihara, ultrastructural features in TEM. A, B. two different cells showing various organelles and structures; C. one complete cell showing details of the two flagella, chloroplasts and mitochondria; D. details of the nucleus (with a possible nucleus blebbing, asterisk), nucleolus, chloroplasts and mitochondria. Af=anterior flagellum, Ch=chloroplasts, M=mitochondria, Mc=mucocysts, N=nucleus, Nu=nucleolus, Pf=posterior flagellum, Py=pyrenoid. All micrographs by David U. Hernández-Becerril.

thylakoids (Fig. 4A, B). We found a maximum of three thylakoids (in bundles) penetrating the pyrenoid (Fig. 4A, B), and empty spaces at opposite extremes, which were interpreted as genophores (Fig. 4B, asterisks).

Mitochondria showed classical tubular cristae (Fig. 4C-E). Vesicles containing fibrous material were additionally found at the anterior part of a cell, close to the external membrane (Fig. 4C), and electron dense material surrounding an almost perfectly rounded empty space (a hole?) or a vesicle (Fig. 4D). The anterior and posterior flagella sections showed the typical structure of 9 + 2 microtubules (Fig. 4E). The mastigonemes (hairs), usually occurring in the anterior flagellum, were not seen. Mucocysts found at the periphery of the cell were observed in detail in a close-up (Fig. 4F, G).

## Phylogeny

One partial LSU rDNA sequence was obtained for *Heterosigma akashiwo*. The final LSU alignment (750 bp) included 61 sequences. Phylogenetic reconstruction from the LSU dataset (with high phylogenetic support; ML/Bi=95/0.96) was performed with sequences of the Class Raphidophyceae (Fig. 5). In the resulting topology, sequences of *H. akashiwo* formed a monophyletic clade with maximum phylogenetic support (ML/Bi=100/1) (Fig. 5). The clade formed by sequences of *H. akashiwo*, grouped sequences of samples collected in different localities of the Atlantic and Pacific Oceans (both in their eastern and western parts) (Fig. 5). Thus, this clade was resolved as a sister clade (with maximum phylogenetic support; ML/Bi=100/1) of the species *Heterosigma minor* (Fig. 5).

The uncorrected genetic distances (p-distances) and corrected genetic distances (the best model) compared between sequences of the species *Heterosigma akashiwo* were 0.0%. However, the calculated genetic distances for species of the order Raphidophyceae ranged from 3.8 to 24.8% (p-distances), and 3.8 to 35.7% for corrected distances based on the best model (Table 1).

## Discussion

### Morphological variation and ultrastructural features

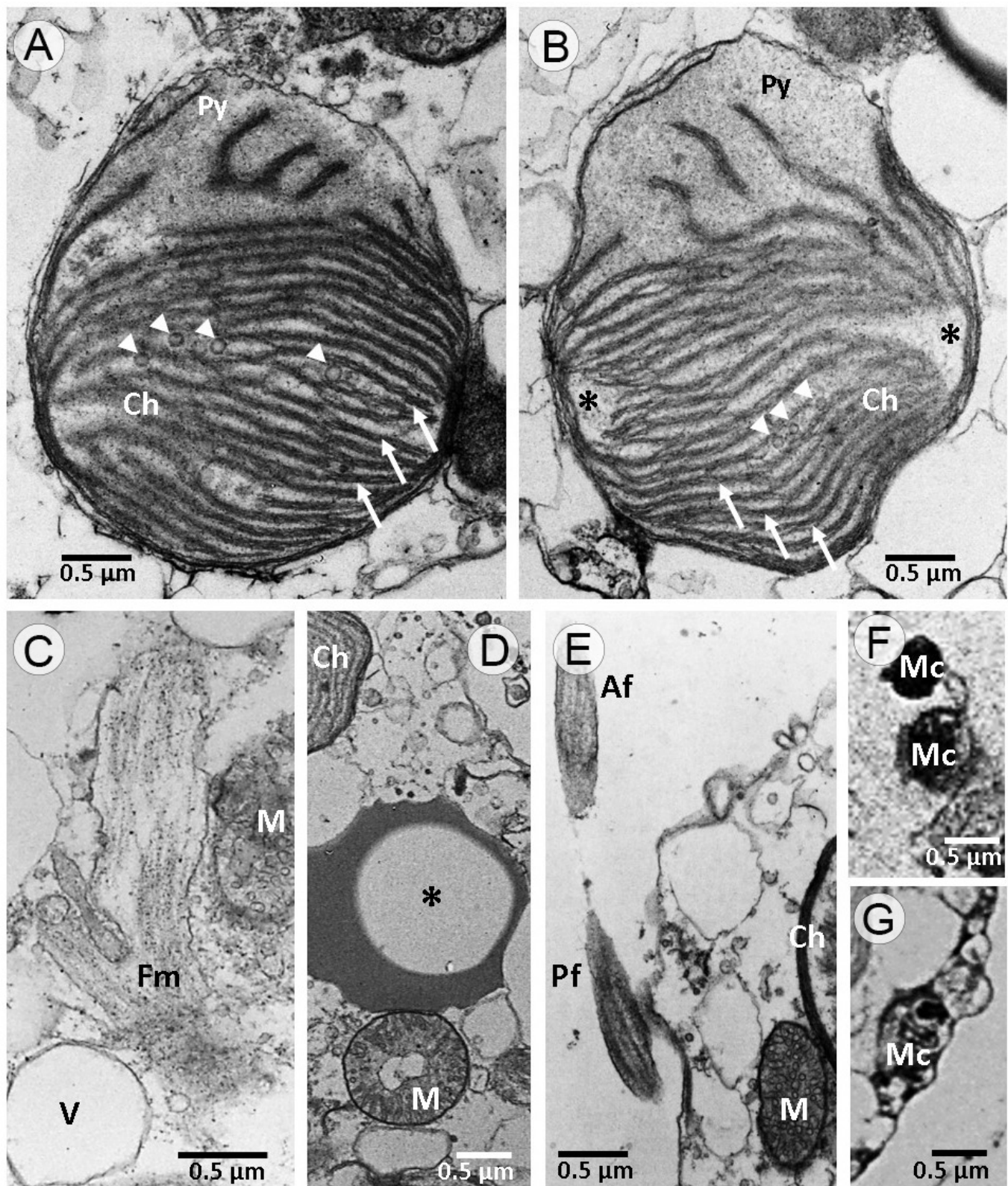
Considerable morphological variability of the species *Heterosigma akashiwo* was found in this study, corresponding with irregular ovoid, oblong and spheroidal cells, with

many brown to yellow chloroplasts in the periphery, previously described (Hara and Chihara, 1987; Fukuyo et al., 1990; Throndsen, 1997; Law and Lee, 2013; Lassus et al., 2016). The shape of the cells has been named as “potato-shaped” cells by some authors (e.g., Hallegraeff and Hara, 2004). This high degree of morphological variation (size and shape of the cells) is usually found in this species all over the world (Hara and Chihara, 1987; Law and Lee, 2013; Branco et al., 2019; Gómez et al., 2022). We herein found and illustrated cells of the species with different size and shapes, which might be the results of both (1) cell stress, with cells distorted under some time under the heat and light of the light microscope, and (2) different stages in culture conditions: actively growing cells, “young” or “aged” cultures (Papini et al., 2017).

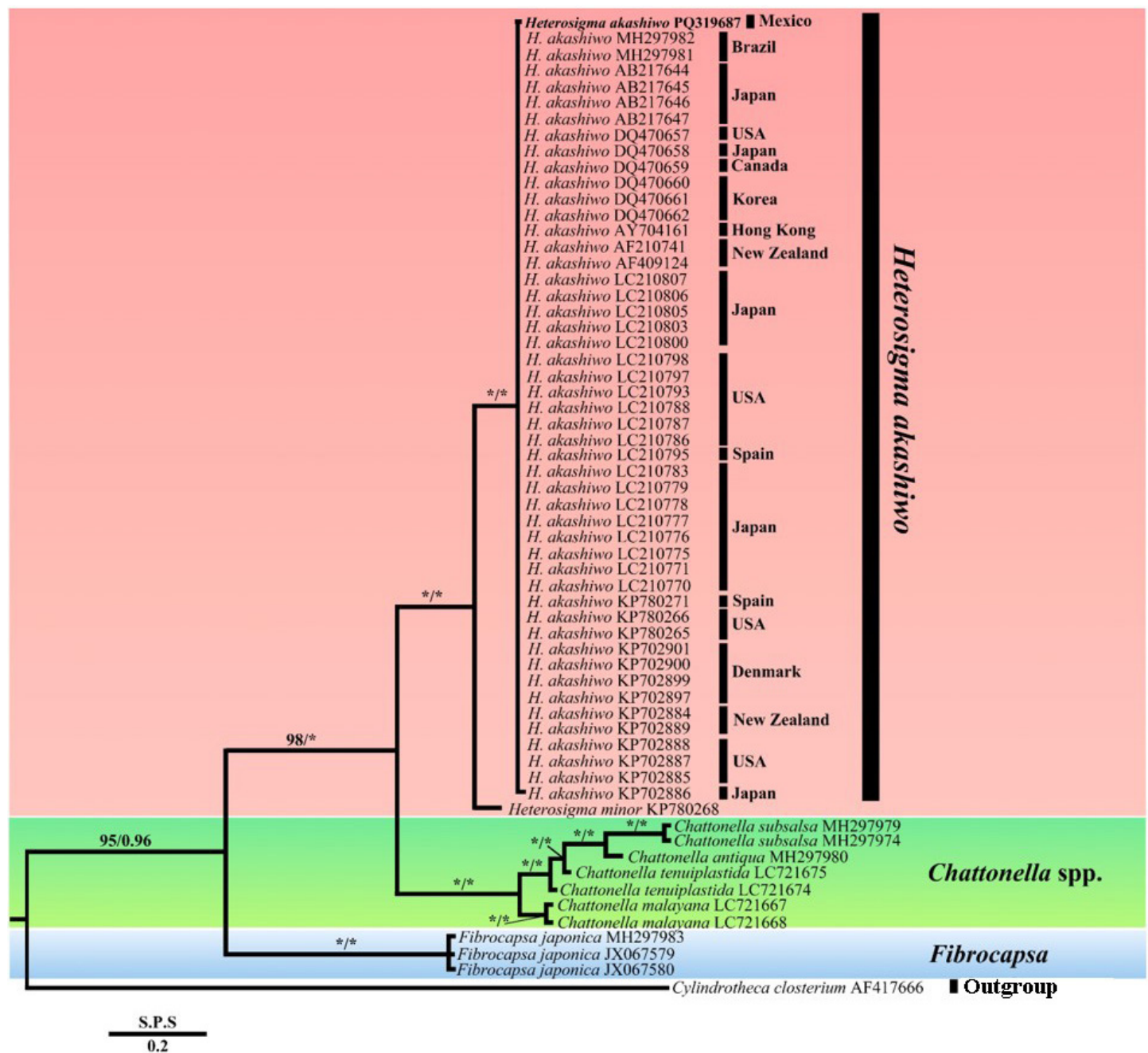
The species can produce resting stages, such as small cells, resting cells and cysts as well as cell clusters (Kim et al., 2023). Resting cells and cysts usually sink to the bottom sediments. Cysts appear mostly spherical, immobile and smaller than the vegetative cells (around 10-15 µm in diameter), yellow-greenish to golden brown in color, with various chloroplasts, and embedded in mucilaginous material (Imai et al., 1993; Itakura et al., 1996; Kim et al., 2015). The life history of the species has been recently studied, showing complex cycles, including cell clusters and homothallic sexual reproduction (Kim et al., 2023). We were unable to find resting stages, cell clusters, and cells undergoing sexual reproduction in our strain.

Studies on the ultrastructure of *Heterosigma akashiwo* made here also showed a previously reported picture about the general structure (shape of cells) and organelles (e.g., nucleus, chloroplasts, mitochondria). However, some differences were also found, such as the size and shape of the nucleus, which was originally observed as a large drop-shaped nucleus in close contact with the flagellar roots (Hara and Chihara, 1987; Fukuyo et al., 1990; Hallegraeff and Hara, 2004). In our study, nuclei were nearly rounded and not very large, although they were located in the center of the cell. Parra et al. (1991) showed a similar situation, with circular to pyriform nuclei. More than one nucleolus has been reported in previous studies (Hara and Chihara, 1987), although we only detected one in one nucleus.





**Figure 4:** *Heterosigma akashiwo* (Hada) Hada ex Y.Hara & Chihara, details of ultrastructural features in TEM. A, B. details of the structure of the chloroplasts, with their pyrenoids, thylakoids (arrows), plastoglobules (arrowheads), and possible genophores (asterisks); C. details of the mitochondria, vacuole and fibrous materials; D. another section showing mitochondria, chloroplast and an empty space (a hole?) or a vesicle (asterisk); E. details of the two flagella, mitochondria and chloroplast; F, G. close-up of the mucocysts in the cell periphery. Af=anterior flagellum, Ch=chloroplasts, Fm=fibrous material, M=mitochondria, Mc=mucocysts, Pf=posterior flagellum, Py=pyrenoid, V=vacuole. All micrographs by David U. Hernández-Becerril.



**Figure 5:** Bayesian inference topology and maximum likelihood based on 28S rDNA (750 bp) sequence data from species of the Raphidophyceae. BI (right) followed by ML bootstrap values (left) on branches. Asterisks indicate full support (ML/BI=100/1). The sequence generated in this study is in boldface. S.P.S.=substitutions per site.

The size and shape of the chloroplasts observed in our study also differed from the almost triangular chloroplasts shown previously for *Heterosigma akashiwo* (Hara and Chihara, 1987; Fukuyo et al., 1990; Parra et al., 1991; Papini et al., 2017). In their ultrastructural study, Branco et al. (2019, fig. 5f) and Orlova et al. (2019) showed more rounded chloroplasts too. We confirm the presence of the

two membranes of the chloroplast envelope and two membranes of the chloroplast endoplasmic reticulum (Larkum and Vesik, 2003). The pyrenoids we observed in our study were not very well-differentiated, because of a reduced matrix, although this reduced matrix was also shown by Branco et al. (2019). In previous papers, pyrenoids were situated at one inner angle of the triangular chloroplasts (Hara and



**Table 1:** Genetic divergence (percentage) between sequences of Raphidophyceae for the 28S rDNA. Uncorrected distances are shown below the diagonal, and Jukes-Cantor distance values are located above the diagonal. Letters highlighted in bold indicate data generated in this study.

|                                                 | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
|-------------------------------------------------|------|------|------|------|------|------|------|
| <b>1.- <i>Heterosigma akashiwo</i> PQ319687</b> | -    | 4.9  | 22.8 | 22.1 | 23.7 | 23.7 | 30.1 |
| 2.- <i>Heterosigma minor</i> KP780268           | 4.8  | -    | 21.9 | 21   | 22.3 | 23   | 29.9 |
| 3.- <i>Chattonella tenuiplastida</i> LC721675   | 19.6 | 19.0 | -    | 6.06 | 3.8  | 4.2  | 34.6 |
| 4.- <i>Chattonella malayana</i> LC721667        | 19.1 | 18.3 | 5.8  | -    | 8.5  | 8.5  | 35.7 |
| 5.- <i>Chattonella subsalsa</i> MH297979        | 20.3 | 19.3 | 3.8  | 8.05 | -    | 5.1  | 34.6 |
| 6.- <i>Chattonella antiqua</i> MH297980         | 20.3 | 19.8 | 4.1  | 8.05 | 4.97 | -    | 34.3 |
| 7.- <i>Fibrocapsa japonica</i> MH297983         | 24.8 | 24.6 | 27.7 | 28.4 | 27.7 | 27.5 | -    |

Chihara, 1987; Papini et al., 2017), whereas we found these structures still orientated towards the centre of the cell. We found structures similar to what was termed genophores by Hara and Chihara (1987) in few chloroplasts.

These differences may be the result of the degree of culture development (exponential growth phase or stationary phase, senescent phases, physiological stages of cells) (Papini et al., 2017; Orlova et al., 2019), but particularly due to various artefacts in the methods followed to observe sections of the cells by TEM, most importantly the fixative procedures, which could cause that some organelles have been slightly enshrined and/or deformed.

We detected tiny and spherical structures among the thylakoids within the chloroplasts, the plastoglobules, which were first shown to be occurring in *Heterosigma akashiwo* by Larkum and Vesk (2003, fig. 6), although they did not mention or notice them. Papini et al. (2017) more recently termed them as plastoglobules, followed by Orlova et al. (2019). Papini et al. (2017) additionally found that the number and electron density of these plastoglobules changed with the age of their cultures, while Orlova et al. (2019) noticed an increase of these structures in stationary phase. Similar structures have also been found in *Chattonella subsalsa* (Branco et al., 2019, fig. 2b), a close relative of *H. akashiwo*. In plants, and most possibly in all photosynthetic organisms, the “plastoglobules function as both lipid biosynthesis and storage subcompartments of thylakoid membranes” (Austin II et al., 2006).

All cells observed in this study belonging to *Heterosigma akashiwo* differed morphologically from the other *Heterosigma* species, its sister *H. minor*, basically in terms

of size and shape. *Heterosigma minor* is smaller (7-11.7 µm length and 6.9-11.5 µm width) and it is more rounded than *H. akashiwo* (10.5-25 µm length and 6-17 µm width) (Engesmo et al., 2016). We recorded 18-26 µm in length and 11-16 µm in width in our specimens of *H. akashiwo*. Additionally, there are 6-14 chloroplasts in *H. minor*, whereas we counted 14-24 per cell. Finally, Engesmo et al. (2016) also mentioned the character of fewer mucocysts in *H. minor* compared with *H. akashiwo*, although we did not observe this feature.

## Phylogeny

The data set analyzed from partial sequences of LSU rDNA in *Heterosigma akashiwo* revealed a monophyletic clade, distributed among different localities around the world. It is important to notice that the set of sequences analyzed for this species did not exhibit divergence at the intraspecific level, which is consistent with the data shown by Engesmo et al. (2016) and Branco et al. (2019). In the topologies shown by Hosoi-Tanabe et al. (2006) and Engesmo et al. (2016), not all the available sequences in GenBank that correspond to *H. akashiwo* were considered. However, in both analyses a polytomy was observed, indicating the presence of a single taxonomic identity. Branco et al. (2019) included a greater number of LSU rDNA sequences from different localities around the world, where a monophyletic clade for *H. akashiwo* was observed. On the other hand, the relationship between the *H. akashiwo* clade and the corresponding subclade of the genus *Chattonella* Biecheler are consistent with that previously reported by Engesmo et al. (2016): both genera are grouped within the family Chattonellaceae. Hi-



gashi et al. (2017) suggested that the mitochondrial genome in *H. akashiwo* could be useful to distinguish strains of this species in distant regions: the use of this highly variable marker would result in an interesting alternative to the LSU rDNA sequences.

### Certain ecological features and distribution in the Mexican Pacific Ocean

*Heterosigma akashiwo* has been a very important species developing harmful blooms in many parts of the world. Some ecological features can provide some adaptive advantages and may be divided in (1) physical conditions, including water column stratification which favors growth of the species (Haigh and Taylor, 1990; Smayda, 1998), and bottom-up controls which may function on mixed-species HAB assemblages (Zhang et al., 2006), and (2) physiological characteristics, for instance its capacity to tolerate a wide range of salinities, or being a euryhaline species (Smayda, 1998; Gómez et al., 2022), its diurnal migrations (Smayda, 1998), and the high level of intraspecific variability within the species: growth rates, pigments and light response curves (Gómez et al., 2022).

Smayda (1997) mentioned “swim” as an advantageous strategy in flagellates, when compared to diatoms, for instance (which use “sink” strategy), and additionally “significant ecophysiological differences”, including mixotrophic tendencies, potential use of allelochemical mechanisms in intraspecific competition and antipredatory defenses, and motility. Fredrickson et al. (2011) have proposed ecotypes (biochemistry, physiology and toxicity) of *Heterosigma akashiwo*, partially explaining several morphological and physiological characteristics such as cell size, maximum growth and photosynthetic rates, tolerance to low salinities, amino acid use and toxicity. Finally, we have to consider the cyst production in *H. akashiwo*, and its role in the bloom formation (Itakura et al., 1996). Natural (and cultural) combinations of hydrographic conditions and ecophysiological features might play an important role in the production of blooms.

*Heterosigma akashiwo* is widely distributed in global waters (Smayda, 1997, 1998; Lassus et al., 2016; Gómez et al., 2022; Mehdizadeh Allaf, 2023). Engesmo et al. (2016) and Mehdizadeh Allaf (2023) have published illustrated

world maps with the global distribution of this species. While it has been commonly reported in the western Pacific Ocean, for most countries in Asia: very often cited in Japan (Hara and Chihara, 1987), and Korea (Lee and Kim, 2008), Hong Kong (Law and Lee, 2013), until Malaysia (Hii et al., 2021), the species is also recorded in the eastern Pacific Ocean, where blooms have historically been recorded in several points and areas along the northeast Pacific Ocean, coasts of Canada, USA and Mexico (Haigh and Taylor, 1990; Horner et al., 1997; O'Halloran et al., 2006; Lewitus et al., 2012; López-Cortés et al., 2016; Rodríguez-Palacio et al., 2019; Anderson et al., 2021), and also in coasts of the southeast Pacific Ocean: Peru (Baylón et al., 2019; Sánchez et al., 2021) and Chile (Parra et al., 1991; Gómez et al., 2022; Mardones et al., 2023). Extreme blooms caused by *H. akashiwo*, reaching 210,000 cells ml<sup>-1</sup>, have been recently recorded in the southern part of Chile (Mardones et al., 2023).

In the Mexican Pacific Ocean, the reports of the species were mentioned above, with no documented cases reporting fish kills (Hernández-Becerril et al., 2007), as these events were produced by blooms of its relatives *Chattonella marina* and *Chattonella marina* cf. *ovata* (Barraza-Guardado et al., 2004; Cortés-Altamirano et al., 2006). Although *Heterosigma akashiwo* was recorded in Acapulco, the tropical Mexican Pacific, by Band-Schmidt et al. (2004), the species was illustrated only by two LM images, with almost no information about their origin. More recent papers did not include reports or illustrations of the species in the area, nor was it included in the harmful algal lists (Meave del Castillo et al., 2012; Meave del Castillo and Zamudio-Reséndiz, 2018; Cervantes-Urieta et al., 2021). This is the first study of the morphology, ultrastructure and molecular phylogeny of the species from the Mexican Pacific, specifically a more tropical area. It is relevant to identify *H. akashiwo* accurately and monitor its populations and dynamics along the Mexican Pacific.

### Author contributions

Conceptualization: DUHB, JAG, AEB; Formal analysis: DUHB, JAG, AEB; Methodology: DUHB, JAG, AEB; Funding acquisition: DUHB; Project administration: DUHB, JAG; Investigation: DUHB, JAG, AEB; Resources: DUHB, JAG, AEB; Writing



- original draft: DUHB; Writing - review and editing: DUHB, JAG, AEB.

## Funding

No special funding was provided, apart from services and facilities mentioned in Acknowledgements.

## Acknowledgements

We are grateful to the Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM) for facilities provided to collect samples. Many thanks and appreciations are also due to Rodolfo Paredes Díaz (Instituto de Fisiología Celular, UNAM) for his technical support in the samples processing for TEM. JAG received a scholarship by the Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT, now Secretaría de Ciencia, Humanidades, Tecnología e Innovación -SECIHTI-) to obtain the M. Sc. degree at the Universidad Autónoma Metropolitana-Iztapalapa.

## Data Accessibility Statement

The dataset supporting the results of this study was submitted to SciELO Data and can be accessed at <https://doi.org/10.48331/SCIELODATA.RJMVP8>.

## Declaration of Artificial Intelligence Use

We declare that the this study was performed without using any AI.

## Literature cited

- Anderson, D. M., E. Fensin, C. J. Gobler, A. E. Hoeglund, K. A. Hubbard, D. M. Kulis, J. H. Landsberg, K. A. Lefebvre, P. Provoost, M. L. Richlen, J. L. Smith, A. R. Solow and V. L. Trainer. 2021. Marine harmful algal blooms (HABs) in the United States: History, current status and future trends. *Harmful Algae* 102: 101975. DOI: <https://doi.org/10.1016/j.hal.2021.101975>
- Austin II, J. R., E. Frost, P.-A. Vidi, F. Kessler and L. A. Staehelin. 2006. Plastoglobules are lipoprotein subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. *The Plant Cell* 18(7): 1693-1703. DOI: <https://doi.org/10.1105/tpc.105.039859>
- Band-Schmidt C. J., A. Martínez-López, J. J. Bustillos-Guzmán, L. Carreón-Palau, L. Morquecho, N. O. Olguín-Monroy, T. Zenteno-Savín, A. Mendoza-Flores, B. González-Acosta, F. H. Hernández-Sandoval and C. Tomas. 2012. Morphology, biochemistry, and growth of raphidophyte strains from the Gulf of California. *Hydrobiologia* 693: 81-97. DOI: <https://doi.org/10.1007/s10750-012-1088-y>
- Band-Schmidt, C. J., A. Martínez-López and I. Gárate-Lizárraga. 2005. First record of *Chattonella marina* in Bahía de La Paz, Gulf of California. *Harmful Algae News* 28: 6-7.
- Band-Schmidt, C. J., L. Morquecho, D. U. Hernández-Becerril, A. Reyes-Salinas and E. Bravo-Sierra. 2004. Raphidophyceans on the coasts of Mexico. *Hydrobiologia* 515: 79-89. DOI: <https://doi.org/10.1023/B:HYDR.0000027320.00977.8b>
- Barraza-Guardado, R., R. Cortés-Altamirano and A. Sierra-Beltrán. 2004. Marine die-offs from *Chattonella marina* and *Ch. cf. ovata* in Kun Kaak Bay, Sonora in the Gulf of California. *Harmful Algae News* 25: 7-8.
- Baylón, M., O. Advíncula, O. Loyola, A. Norabuena and D. U. Hernández-Becerril. 2019. Variación espacial del fitoplancton con énfasis en las floraciones algales frente a la playa de pescadores artesanales de Chorrillos/Lima/Perú. *Ecología Aplicada* 18(2): 133-143. DOI: <https://doi.org/10.21704/rea.v18i2.1332>
- Benico, G., K. Takahashi, W. M. Lum and M. Iwataki. 2019. Morphological variation, ultrastructure, pigment composition and phylogeny of the star-shaped dinoflagellate *Asterodinium gracile* (Kareniaceae, Dinophyceae). *Phycologia* 58(4): 405-418. DOI: <https://doi.org/10.1080/00318884.2019.1601948>
- Branco, S., L. L. Almeida, C. Alves-de-Souza, M. M. M. Oliveira, L. A. O. Proença and M. Menezes. 2019. Morphological and genetic characterization of bloom-forming Raphidophyceae from Brazilian coast. *Phycological Research* 67(4): 279-290. DOI: <https://doi.org/10.1111/pre.12377>
- Bravo-Sierra, E. 2004. Fitoflagelados potencialmente tóxicos y nocivos de costas del Pacífico mexicano. *Revista de Biología Tropical* 52(Suppl. 1): 5-16.
- Cervantes-Urieta, V. A., M. N. Trujillo-Tapia, J. Violante-González, G. Moreno-Díaz, A. A. Rojas-Herrera and V. Rosas-Guerrero. 2021. Temporal dynamics of the phytoplankton community associated with environmental factors and harmful algal blooms in Acapulco Bay, Mexico. *Latin American Journal of Aquatic Research* 49(1): 110-124.



- Chang, F. H., C. Anderson and N. C. Boustead. 1990. First record of a *Heterosigma* (Raphidophyceae) bloom with associated mortality of cage-reared salmon in Big Glory Bay, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 24(4): 461-469. DOI: <https://doi.org/10.1080/00288330.1990.9516437>
- Cortés-Altamirano, R., R. Alonso-Rodríguez and A. Sierra-Beltrán. 2006. Fish mortality associated with *Chattonella marina* and *Ch. cf. ovata* (Raphidophyceae) blooms in Sinaloa (Mexico). *Harmful Algae News* 31: 7-8.
- Cortés-Lara, M. C., R. Cortés-Altamirano and A. Cupul-Magaña. 2003. First record of *Fibrocapsa cf. japonica* in Matanchen Bay, Nayarit, Mexican Pacific coast. *Harmful Algae News* 24: 1-4.
- Dorantes-Aranda, J. J., A. Seger, J. I. Mardones, P. D. Nichols, and G. M. Hallegraeff. 2015. Progress in understanding algal bloom-mediated fish kills: the role of superoxide radicals, phycotoxins and fatty acids. *PLoS One* 10(7): e0133549. DOI: <https://doi.org/10.1371/journal.pone.0133549>
- Engesmo, A., W. Eikrem, S. Seoane, K. Smith, B. Edvardsen, A. Hofgaard and C.R. Tomas. 2016. New insights into the morphology and phylogeny of *Heterosigma akashiwo* (Raphidophyceae), with the description of *Heterosigma minor* sp. nov. *Phycologia* 55(3): 279-294. DOI: <https://doi.org/10.2216/15-115.1>
- Escarcega-Bata, A., M. C. Ruiz-de la Torre, M. L. Nuñez-Reséndiz, L. M. Enriquez-Paredes, K. M. Dreckmann and A. Senties. 2021. Molecular assessment of athecate dinoflagellates of the order Gymnodiniales (Dinophyceae) in Todos Santos Bay, Baja California México. *American Journal of Plant Sciences* 12(12): 1926-1944. DOI: <https://doi.org/10.4236/ajps.2021.1212133>
- Flores-Leñero, A., V. Vargas-Torres, J. Paredes-Mella, L. Norambuena, G. Fuenzalida, K. Lee-Chang and J.I. Mardones. 2022. *Heterosigma akashiwo* in Patagonian Fjords: genetics, growth, pigment signature and role of PUFA and ROS in ichthyotoxicity. *Toxins* 14(9): 577. DOI: <https://doi.org/10.3390/toxins14090577>
- Fredrickson, K. A., S. L. Strom, R. Crim and K. J. Coyne. 2011. Interstrain variability in physiology and genetics of *Heterosigma akashiwo* (Raphidophyceae) from the west coast of North America. *Journal of Phycology* 47(1): 25-35. DOI: <https://doi.org/10.1111/j.1529-8817.2010.00942.x>
- Fukuyo, Y., H. Takano, M. Chihara and K. Matsuoka. 1990. Red tide organisms in Japan - An Illustrated Taxonomic Guide. Uchida Rokakuho. Tokyo, Japan. 430 pp.
- Gárate-Lizárraga, I., C. J. Band-Schmidt, D. J. López-Cortés and J. J. Bustillos-Guzmán. 2009. Raphidophytes in Bahía de La Paz, Gulf of California. *Harmful Algae News* 40: 1-4.
- Gómez, P. I., I. Inostroza, P. Castro-Varela, J. Silva, A. Clément, G. Rojas, A. Aguilera Belmonte. 2022. Comparison of a Chilean strain of the ichthyotoxic phytoflagellate *Heterosigma akashiwo* (Raphidophyceae) with strains from France, Spain and New Zealand. *Phycologia* 61(1): 7-15. DOI: <https://doi.org/10.1080/00318884.2021.1991685>
- Haigh, R. and F. J. R. Taylor. 1990. Distribution of potentially harmful phytoplankton species in the northern Strait of Georgia, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 2339-2358.
- Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.
- Hallegraeff, G. M., and Y. Hara. 2004. Taxonomy of harmful marine raphidophytes. In: Hallegraeff, G.M., D.M. Anderson and A. Cembella (eds.). *Manual on harmful marine microalgae*, 2nd ed. United Nations Educational, Scientific and Cultural Organization (UNESCO). Paris, France. Pp 511-522.
- Hara, Y. and M. Chihara. 1987. Morphology, ultrastructure and taxonomy of the raphidophycean alga *Heterosigma akashiwo*. *The Botanical Magazine = Shokubutsu-gaku-zasshi* 100: 151-163. DOI: <https://doi.org/10.1007/BF02488320>
- Hernández-Becerril, D. U. 2014. Biodiversidad de algas planctónicas marinas (Cyanobacteria, Prasinophyceae, Euglenophyta, Chrysophyceae, Dictyochophyceae, Eustigmatophyceae, Parmophyceae, Raphidophyceae, Bacillariophyta, Cryptophyta, Haptophyta, Dinoflagellata) en México. *Revista Mexicana de Biodiversidad* 85: S44-S53. DOI: <https://doi.org/10.7550/rmb.32037>
- Hernández-Becerril, D. U. and E. Bravo-Sierra. 1999. Presencia del fitoflagelado planctónico *Heterosigma akashiwo* (Raphidophyceae) en costas de México. In: X Reunión Nacional de la Sociedad Mexicana de Planctología, A.C. and II Reunión Internacional de Planctología. 28-30 abril 1999. Mazatlán, Sinaloa, México. Pp. 9-10.
- Hernández-Becerril, D.U., R. Alonso-Rodríguez, C. Álvarez-Góngora, S. A. Barón-Campis, G. Ceballos-Corona, J. Herrera-



- Silveira, M.E. Meave del Castillo, N. Juárez-Ruiz, F. Merino-Virgilio, A. Morales-Blake, J.L. Ochoa, E. Orellana-Cepeda, C. Ramírez-Camarena and R. Rodríguez-Salvador. 2007. Toxic and harmful marine phytoplankton and microalgae (HABs) in Mexican coasts. *Journal of Environmental Science and Health, Part A* 42(10): 1349-1363. DOI: <https://doi.org/10.1080/10934520701480219>
- Higashi, A., S. Nagai, P. S. Salomon and S. Ueki. 2017. A unique, highly variable mitochondrial gene with coding capacity of *Heterosigma akashiwo*, class Raphidophyceae. *Journal of Applied Phycology* 29: 2961-2969. DOI: <https://doi.org/10.1007/s10811-017-1142-2>
- Hii, K. S., M. Mohd-Din, Z. Luo, S. N. Tan, Z. F. Lim, L. K. Lee, S. C. Y. Leong, S. T. Teng, H. Gu, X. Cao, P. T. Lim and C. P. Leaw. 2021. Diverse harmful microalgal community assemblages in the Johor Strait and the environmental effects on its community dynamics. *Harmful Algae* 107: 102077. DOI: <https://doi.org/10.1016/j.hal.2021.102077>
- Horiguchi, T. 2017. Raphidophyceae (Raphidophyta). In: Archibald, J. M., A. G. B. Simpson and C. H. Slamovits (eds.). *Handbook of the protists*, 2nd ed. Springer. Cham, Swiss. Pp. 305-330. DOI: [https://doi.org/10.1007/978-3-319-32669-6\\_37-1](https://doi.org/10.1007/978-3-319-32669-6_37-1)
- Horner, R. A., D. L. Garrison and F. G. Plumley. 1997. Harmful algal blooms and red tide problems on the U.S. west coast. *Limnology and Oceanography* 42(5): 1076-1088. DOI: [https://doi.org/10.4319/lo.1997.42.5\\_part\\_2.1076](https://doi.org/10.4319/lo.1997.42.5_part_2.1076)
- Hosoi-Tanabe, S., I. Otake and Y. Sako. 2006. Phylogenetic analysis of noxious red tide flagellates *Chattonella antiqua*, *C. marina*, *C. ovata* and *C. verruculosa* (Raphidophyceae) based on the rRNA gene family. *Fisheries Science* 72: 1200-1208. DOI: <https://doi.org/10.1111/j.1444-2906.2006.01277.x>
- Imai, I., S. Itakura and K. Itoh. 1993. Cysts of the red tide flagellate *Heterosigma akashiwo*, Raphidophyceae, found in bottom sediments of northern Hiroshima Bay, Japan. *Bulletin of the Japanese Society of Scientific Fisheries-Nippon Suisan Gakkaishi* 59(10): 1669-1673. DOI: <https://doi.org/10.2331/suisan.59.1669>
- Itakura, S., K. Nagasaki, M. Yamaguchi and I. Imai. 1996. Cyst formation in the red tide flagellate *Heterosigma akashiwo* (Raphidophyceae). *Journal of Plankton Research* 18(10): 1975-1979. DOI: <https://doi.org/10.1093/plankt/18.10.1975>
- Jeffrey, S. W. and M. Vesik. 1997. Introduction to marine phytoplankton and their pigment signatures. In: Jeffrey, S.W., R.F.C. Mantoura and S. W. Wright (eds.). *Phytoplankton pigments in oceanography: guidelines to modern methods*. United Nations Educational, Scientific and Cultural Organization (UNESCO). Paris, France. Pp. 37-84.
- Jeong, H. J. 2011. Mixotrophy in red tide algae raphidophytes. *The Journal of Eukaryotic Microbiology* 58(3): 215-222. DOI: <https://doi.org/10.1111/j.1550-7408.2011.00550.x>
- Khan, S., O. Arakawa and Y. Onoue. 1997. Neurotoxins in a toxic red tide of *Heterosigma akashiwo* (Raphidophyceae) in Kagoshima Bay, Japan. *Aquaculture Research* 28: 9-14. DOI: <https://doi.org/10.1046/j.1365-2109.1997.t01-1-00823.x>
- Kim, J. H., B. S. Park and J. H. Kim. 2023. Comprehensive understanding of the life history of harmful raphidophyte *Heterosigma akashiwo*: Integrating in situ and in vitro observations. *Harmful Algae* 129: 102521. DOI: <https://doi.org/10.1016/j.hal.2023.102521>
- Kim, J.-H., B. S. Park, P. Wang, S. H. Youn and M.-S. Han. 2015. Cyst morphology and germination in *Heterosigma akashiwo* (Raphidophyceae). *Phycologia* 54(5): 435-439. DOI: <https://doi.org/10.2216/15-26.1>
- Larkum, A. W. D. and M. Vesik. 2003. Algal plastids: their fine structure and properties. In: Larkum, A. W. D., S. E. Douglas and J. A. Raven (eds.). *Photosynthesis in algae. Advances in Photosynthesis and Respiration*, vol 14. Springer. Dordrecht, The Netherlands. Pp. 11-28. DOI: [https://doi.org/10.1007/978-94-007-1038-2\\_2](https://doi.org/10.1007/978-94-007-1038-2_2)
- Lassus, P., N. Chomérat, P. Hess and E. Nézan. 2016. Toxic and harmful microalgae of the world ocean/Micro-algues toxiques et nuisibles de l'océan mondial. *International Society for the Study of Harmful Algae, Intergovernmental Oceanographic Commission of UNESCO. IOC Manuals and Guides* 68. Odense, Denmark. 523 pp.
- Law, S. P. C. and F. Y. K. Lee. 2013. *Harmful marine microalgae in Hong Kong*. Agriculture, fisheries and conservation department. Hong Kong SAR, China. 114 pp.
- Lee, M. O. and J. K. Kim. 2008. Characteristics of algal blooms in the southern coastal waters of Korea. *Marine Environmental Research* 65(2): 128-147. DOI: <https://doi.org/10.1016/j.marenvres.2007.09.006>
- Lewitus, A. J., R. A. Horner, D. A. Caron, E. Garcia-Mendoza, B. M. Hickey, M. Hunter, D. Huppert, R. M. Kudela, G. W. Langlois, J. L. Largier, E. Lessard, R. RaLonde, J. Rensel, P. Strutton, V. L. Trainer and J. F. Tweddle. 2012. Harmful algal blooms



- along the North American west coast region: History, trends, causes, and impacts. *Harmful Algae* 19: 133-159. DOI: <https://doi.org/10.1016/j.hal.2012.06.009>
- López-Cortés, D. J., F. E. Hernández-Sandoval, C. J. Band-Schmidt, J. J. Bustillos-Guzmán and E. J. Núñez-Vázquez. 2016. Condiciones ambientales asociadas a florecimientos algales nocivos en el Golfo de California. In: García-Mendoza, E., S. I. Quijano-Scheggia, A. Olivos-Ortiz and E. J. Núñez-Vázquez (eds.). *Florecimientos algales nocivos en México*. Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, México. Pp. 146-161.
- Lum, W. M., H. C. Lim, W. L. S. Lau, I. K. Law, S. T. Teng, G. Benico, S. C. Y. Leong, K. Takahashi, H. Gu, T. Lirdwitayaprasit, C. P. Leaw, P. T. Lim, M. Iwataki. 2022. Description of two new species *Chattonella tenuiplastida* sp. nov. and *Chattonella malayana* sp. nov. (Raphidophyceae) from South China Sea, with a report of wild fish mortality. *Harmful Algae* 118: 102322. DOI: <https://doi.org/10.1016/j.hal.2022.102322>
- Mardones, J. I., J. Paredes-Mella, A. Flores-Leñero, K. Yarimizu, M. Godoy, O. Artal, A. Corredor-Acosta, L. Marcus, E. Cascales, J. P. Espinoza, L. Norambuena, R. D. Garreaud, H. E. González and J. L. Iriarte. 2023. Extreme harmful algal blooms, climate change, and potential risk of eutrophication in Patagonian fjords: Insights from an exceptional *Heterosigma akashiwo* fish-killing event. *Progress in Oceanography* 210: 102921. DOI: <https://doi.org/10.1016/j.pocean.2022.102921>
- Marshall, J.-A., M. de Salas, T. Oda, G. M. Hallegraeff. 2005. Superoxide production by marine microalgae. I. Survey of 37 species from 6 classes. *Marine Biology* 147: 533-540. DOI: <https://doi.org/10.1007/s00227-005-1596-7>
- Martínez-López, A., C. J. Band-Schmidt, D. Escobedo-Urías and A. E. Ulloa. 2006. Bloom of *Chattonella subsalsa* in an impacted coastal lagoon in the Gulf of California. *Harmful Algae News* 31: 4-5.
- Meave del Castillo, M. E. and M. E. Zamudio-Reséndiz. 2018. Florecimientos de microalgas planctónicas de 2000 al 2015 en la Bahía de Acapulco, Guerrero, México. *Acta Botanica Mexicana* 125: 61-93. DOI: <https://doi.org/10.21829/abm125.2018.1316>
- Meave del Castillo, M. E., M. E. Zamudio-Reséndiz and M. Castillo-Rivera. 2012. Riqueza fitoplanctónica de la Bahía de Acapulco y zona costera aledaña, Guerrero, México. *Acta Botanica Mexicana* 100: 405-487. DOI: <https://doi.org/10.21829/abm100.2012.41>
- Mehdizadeh Allaf, M. 2023. *Heterosigma akashiwo*, a fish-killing flagellate. *Microbiological Research* 14(1): 132-147. DOI: <https://doi.org/10.3390/microbiolres14010012>
- O'Halloran, C., M. W. Silver, T. R. Holman and C. A. Scholin. 2006. *Heterosigma akashiwo* in central California waters. *Harmful Algae* 5(2): 124-132. DOI: <https://doi.org/10.1016/j.hal.2005.06.009>
- Orlova, T. Yu., M. A. Sabutskaya and Zh. V. Markina. 2019. Ultrastructural changes in marine microalgae from different taxonomic groups during batch cultivation. *Russian Journal of Marine Biology* 45: 202-210. DOI: <https://doi.org/10.1134/S1063074019030106>
- Papini, A., F. Fani, M. Belli, C. Niccolai, C. Tani, P. Di Falco, C. Nuccio and L. Lazzara. 2017. Structural and ultrastructure changes show an increase in amoeboid forms in *Heterosigma akashiwo* (Raphidophyceae), during recovery after nutrient depletion. *Plant Biosystems* 151(6): 965-973. DOI: <https://doi.org/10.1080/11263504.2016.1218970>
- Parra, O. O., P. R. Rivera, G. L. Floyd and L. W. Wilcox. 1991. Cultivo, morfología, ultraestructura y taxonomía de un fitoflagelado asociado a mareas rojas en Chile: *Heterosigma akashiwo* (Hada) Hada. *Gayana Botánica* 48(1-4): 101-110.
- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25(7): 1253-1256. DOI: <https://doi.org/10.1093/molbev/msn083>
- Rodríguez-Palacio, M., C. Lozano-Ramírez and S. H. Álvarez-Hernández. 2019. HABs (harmful algal blooms) analysis, their cost, and ecological consequences. In: Ibáñez, A.L. (ed.). *Mexican aquatic environments. A general view from hydrobiology to fisheries*. Springer Nature. Cham, Switzerland. Pp. 219-263. DOI: [https://doi.org/10.1007/978-3-030-11126-7\\_10](https://doi.org/10.1007/978-3-030-11126-7_10)
- Ronquist, F., M. Teslenko, P. Van Der Mar, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542. DOI: <https://doi.org/10.1093/sysbio/sys029>
- Sánchez, S., E. Delgado, A. Bernal, N. Jacobo, A. Franco and D. Correa. 2021. Floraciones algales nocivas en la costa peruana durante El Niño Costero 2017 y su relación con las



- condiciones ambientales. Boletín Instituto del Mar del Perú 36(2): 452-462. DOI: <https://doi.org/10.53554/boletin.v36i2.347>
- Scholin, C. A., M. Herzog, M. Sogin and D. M. Anderson. 1994. Identification of group and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the large subunit ribosomal RNA gene. Journal of Phycology 30(6): 999-1011. DOI: <https://doi.org/10.1111/j.0022-3646.1994.00999.x>
- Smayda, T. J. 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 45(5 part 2): 1137-1153. DOI: [https://doi.org/10.4319/lo.1997.42.5\\_part\\_2.1137](https://doi.org/10.4319/lo.1997.42.5_part_2.1137)
- Smayda, T. J. 1998. Ecophysiology and bloom dynamics of *Heterosigma akashiwo* (Raphidophyceae). In Anderson, D.M., A. D. Cembella and G. M. Hallegraeff (eds.). Physiological ecology of harmful algal blooms. Springer-Verlag, Berlin, Germany. Pp. 113-131.
- Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21): 2688-2690. DOI: <https://doi.org/10.1093/bioinformatics/btl446>
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731-2739. DOI: <https://doi.org/10.1093/molbev/msr121>
- Thronsdon, J. 1997. The planktonic marine flagellates. In: Tomas, C. R. (ed.). Identifying marine phytoplankton. Academic Press, San Diego, USA. Pp. 591-729.
- Twiner, M. J. and C. G. Trick. 2000. Possible physiological mechanisms for production of hydrogen peroxide by the ichthyotoxic flagellate *Heterosigma akashiwo*. Journal of Plankton Research 22(10): 1961-1975. DOI: <https://doi.org/10.1093/plankt/22.10.1961>
- Yang, C. Z., L. J. Albright and A. N. Yousif. 1995. Oxygen-radical-mediated effects of the toxic phytoplankter *Heterosigma carterae* on juvenile rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms 23: 101-108. DOI: <https://doi.org/10.3354/dao023101>
- Zhang, Y. H., F. X. Fu, E. Whereat, K. J. Coyne and D. A. Hutchins. 2006. Bottom-up controls on a mixed-species HAB assemblage: a comparison of sympatric *Chattonella subsalsa* and *Heterosigma akashiwo* (Raphidophyceae) isolates from the Delaware Inland Bays, USA. Harmful Algae 5(3): 310-320. DOI: <https://doi.org/10.1016/j.hal.2005.09.001>



**Appendix:** Large subunit (LSU) rDNA sequences used for phylogenetic analysis, including geographic information and GenBank accession numbers. The GenBank accession number in boldface corresponds to the sequence generated in this study. The analysis included a total of eight species.

| Taxa                                                                         | Distribution data           | GenBank accession number |
|------------------------------------------------------------------------------|-----------------------------|--------------------------|
| <i>Cylindrotheca closterium</i> (Ehrenberg) Reimann & Lewin                  | Kattegat, Denmark           | AF417666                 |
| <i>Chattonella tenuiplastida</i> Lum, Lim, Takahashi, Teng, Benico & Iwataki | Ko Chang, Thailand          | LC721674                 |
| <i>Chattonella tenuiplastida</i>                                             | Ko Chang, Thailand          | LC721675                 |
| <i>Chattonella malayana</i> Lum, Lim, Teng, Takahashi, Leaw, Lim & Iwataki   | Pantai Melawi, Malaysia     | LC721667                 |
| <i>C. malayana</i>                                                           | Pantai Melawi, Malaysia     | LC721668                 |
| <i>Chattonella subsalsa</i> Biecheler                                        | Guanabara Bay, Brazil       | MH297979                 |
| <i>C. subsalsa</i>                                                           | Guanabara Bay, Brazil       | MH297974                 |
| <i>Chattonella antiqua</i> (Hada) Ono                                        | South Bay, Brazil           | MH297980                 |
| <i>Fibrocapsa japonica</i> Toriumi & Takano                                  | Pereque Beach, Brazil       | MH297983                 |
| <i>F. japonica</i>                                                           | North Sea, Germany          | JX067579                 |
| <i>F. japonica</i>                                                           | Port Phillip Bay, Australia | JX067580                 |
| <b><i>Heterosigma akashiwo</i> (Hada) Hada ex Hara &amp; Chihara</b>         | <b>Acapulco Bay, Mexico</b> | <b>PQ319687</b>          |
| <i>H. akashiwo</i>                                                           | São Miguel Beach, Brazil    | MH297982                 |
| <i>H. akashiwo</i>                                                           | Guanabara Bay, Brazil       | MH297981                 |
| <i>H. akashiwo</i>                                                           | Gokasyo Bay, Japan          | AB217644                 |
| <i>H. akashiwo</i>                                                           | Nomaike, Japan              | AB217645                 |
| <i>H. akashiwo</i>                                                           | Hiroshima Bay, Japan        | AB217646                 |
| <i>H. akashiwo</i>                                                           | Japan                       | AB217647                 |
| <i>H. akashiwo</i>                                                           | Long Island, USA            | DQ470657                 |
| <i>H. akashiwo</i>                                                           | Onagawa Bay, Japan          | DQ470658                 |
| <i>H. akashiwo</i>                                                           | Vancouver, Canada           | DQ470659                 |
| <i>H. akashiwo</i>                                                           | South Korea                 | DQ470660                 |
| <i>H. akashiwo</i>                                                           | South Korea                 | DQ470661                 |
| <i>H. akashiwo</i>                                                           | Masan Bay, South Korea      | DQ470662                 |
| <i>H. akashiwo</i>                                                           | Hong Kong, China            | AY704161                 |
| <i>H. akashiwo</i>                                                           | New Zealand                 | AF210741                 |
| <i>H. akashiwo</i>                                                           | New Zealand                 | AF409124                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210807                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210806                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210805                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210803                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210800                 |
| <i>H. akashiwo</i>                                                           | USA                         | LC210798                 |
| <i>H. akashiwo</i>                                                           | USA                         | LC210797                 |
| <i>H. akashiwo</i>                                                           | Spain                       | LC210795                 |
| <i>H. akashiwo</i>                                                           | USA                         | LC210793                 |
| <i>H. akashiwo</i>                                                           | USA                         | LC210788                 |
| <i>H. akashiwo</i>                                                           | USA                         | LC210787                 |
| <i>H. akashiwo</i>                                                           | USA                         | LC210786                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210783                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210779                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210778                 |
| <i>H. akashiwo</i>                                                           | Japan                       | LC210777                 |



## Appendix: Continuation.

| Taxa                                             | Distribution data | GenBank accession number |
|--------------------------------------------------|-------------------|--------------------------|
| <i>H. akashiwo</i>                               | Japan             | LC210776                 |
| <i>H. akashiwo</i>                               | Japan             | LC210775                 |
| <i>H. akashiwo</i>                               | Japan             | LC210771                 |
| <i>H. akashiwo</i>                               | Japan             | LC210770                 |
| <i>H. akashiwo</i>                               | Spain             | KP780271                 |
| <i>H. akashiwo</i>                               | USA               | KP780266                 |
| <i>H. akashiwo</i>                               | USA               | KP780265                 |
| <i>H. akashiwo</i>                               | Denmark           | KP702901                 |
| <i>H. akashiwo</i>                               | Denmark           | KP702900                 |
| <i>H. akashiwo</i>                               | Denmark           | KP702899                 |
| <i>H. akashiwo</i>                               | New Zealand       | KP702894                 |
| <i>H. akashiwo</i>                               | New Zealand       | KP702897                 |
| <i>H. akashiwo</i>                               | New Zealand       | KP702889                 |
| <i>H. akashiwo</i>                               | USA               | KP702888                 |
| <i>H. akashiwo</i>                               | USA               | KP702887                 |
| <i>H. akashiwo</i>                               | Japan             | KP702886                 |
| <i>H. akashiwo</i>                               | USA               | KP702885                 |
| <i>Heterosigma minor</i> Tomas, Engesmo & Eikrem | USA               | KP780268                 |

